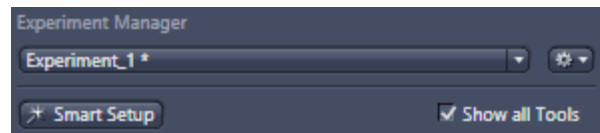


This guide will outline the steps necessary to create an image of a tiled region (mosaic). The main steps include:

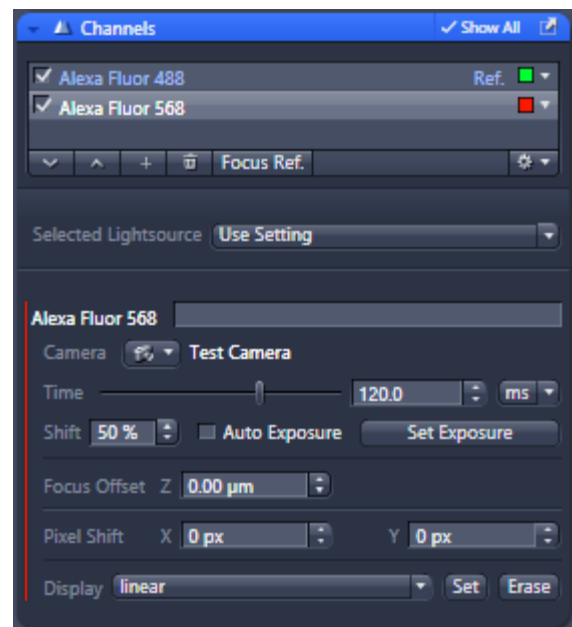
1. Loading an experiment, optimizing camera settings and setting shading correction
2. Using the Advanced Setup in the Tiles Window to locate the edges or “landmarks” of your sample and creating the Tile Region
3. Adding Support Points to determine the Local Focus Surface, or interpolated focusing plane
4. Activating the Focus Strategy and acquiring the tiled image
5. Stitching of the tiled image

### 1. Loading an experiment, optimizing camera settings and activating shading correction

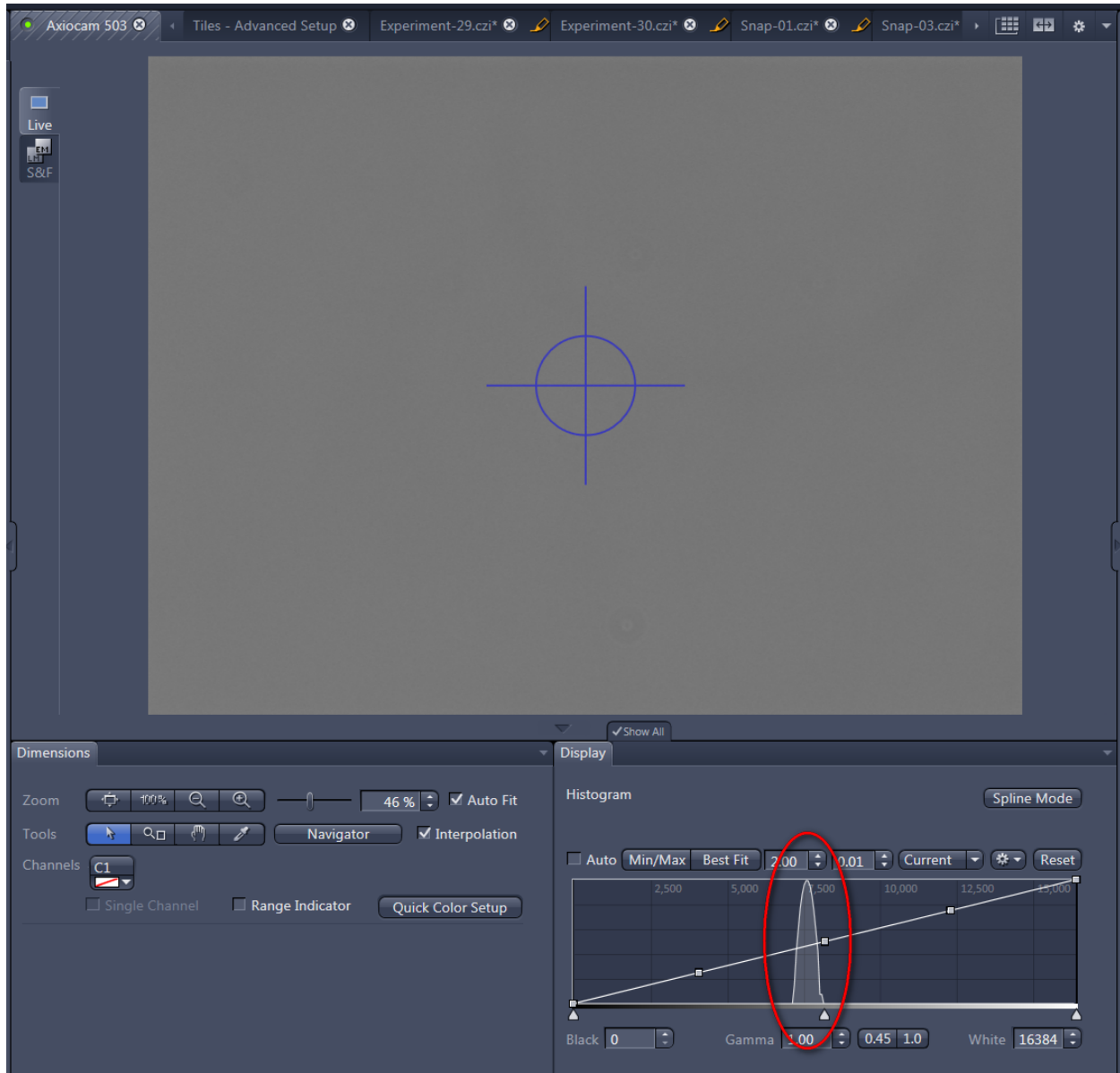
Create a new experiment using the “Smart Setup” or load an existing experiment to populate the “Channels” window with the appropriate fluorescence or transmitted light channels.



Adjust the camera exposure values and/or light intensity for each channel using the “Live” window.



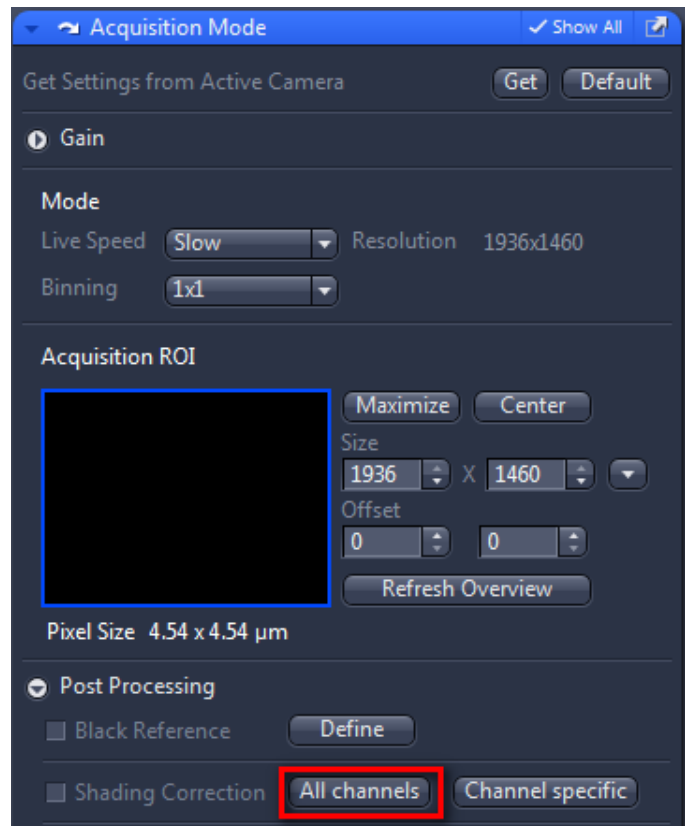
In addition to setting proper Koehler illumination, it is recommended that you also set a shading correction reference image to eliminate vignetting effects while tiling using transmitted light. It should not be necessary to perform this step while acquiring fluorescence channels.



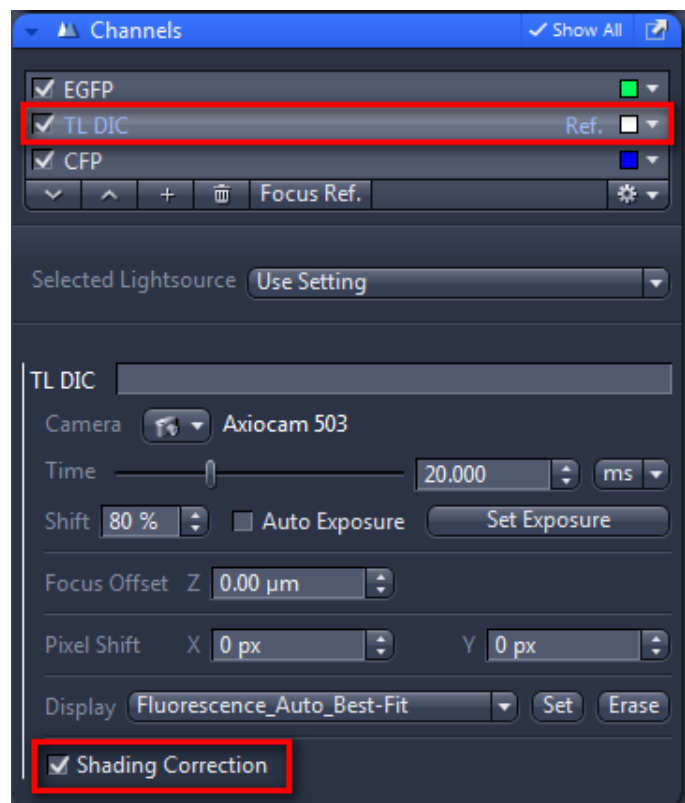
Start by opening a live window and choose a transmitted light channel. Move the stage to an area within the slide devoid of any sample or debris. Defocus a bit if there is debris that can contaminate the reference image. If you cannot find a clean region, move the stage totally off the sample slide.

Set the voltage of the transmitted light lamp such that the peak intensity in the histogram is somewhere around the middle of the dynamic range of the camera.

Open the “Acquisition mode” window and expand the “Post Processing” subsection. Press the “All Channels” button under “Shading Correction” to set the shading reference image. The checkbox will automatically check itself and the live window should look more homogeneous than before. Close the live window.

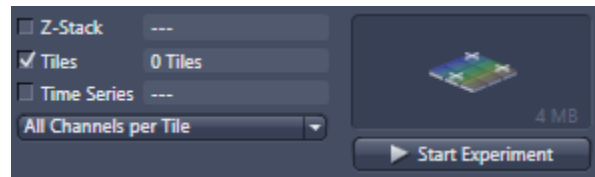


In the “Channels” window, highlight the transmitted light channel and check the “Shading Correction” box to apply the shading reference image as the images are acquired during the experiment.



## 2. Using “Advanced Setup” to create the Tile Region(s)

Activate the “Tiles” checkbox in the multidimensional acquisition pane.

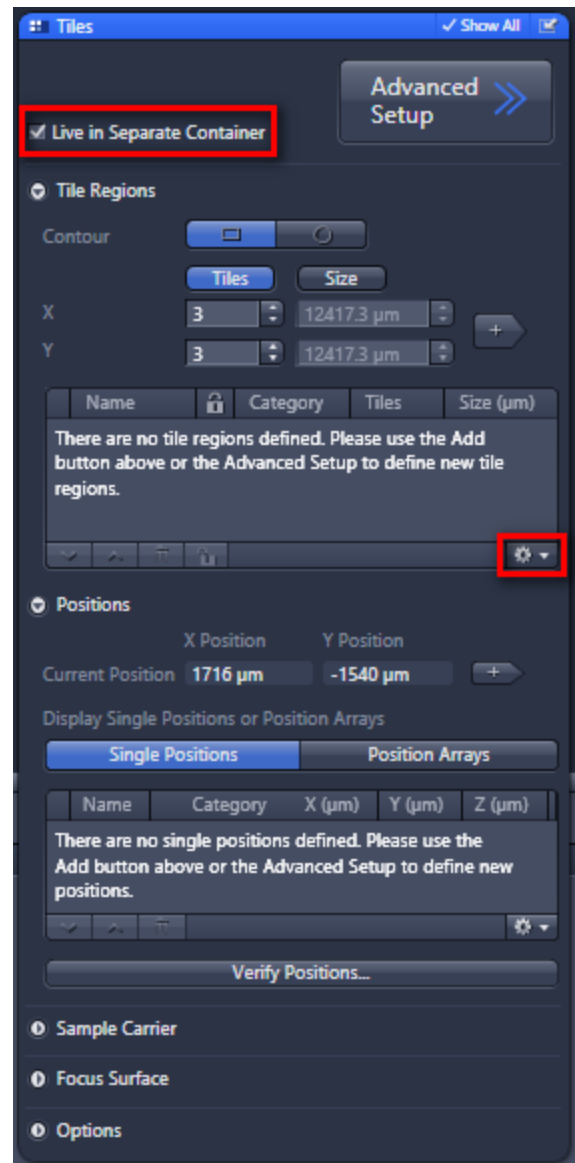


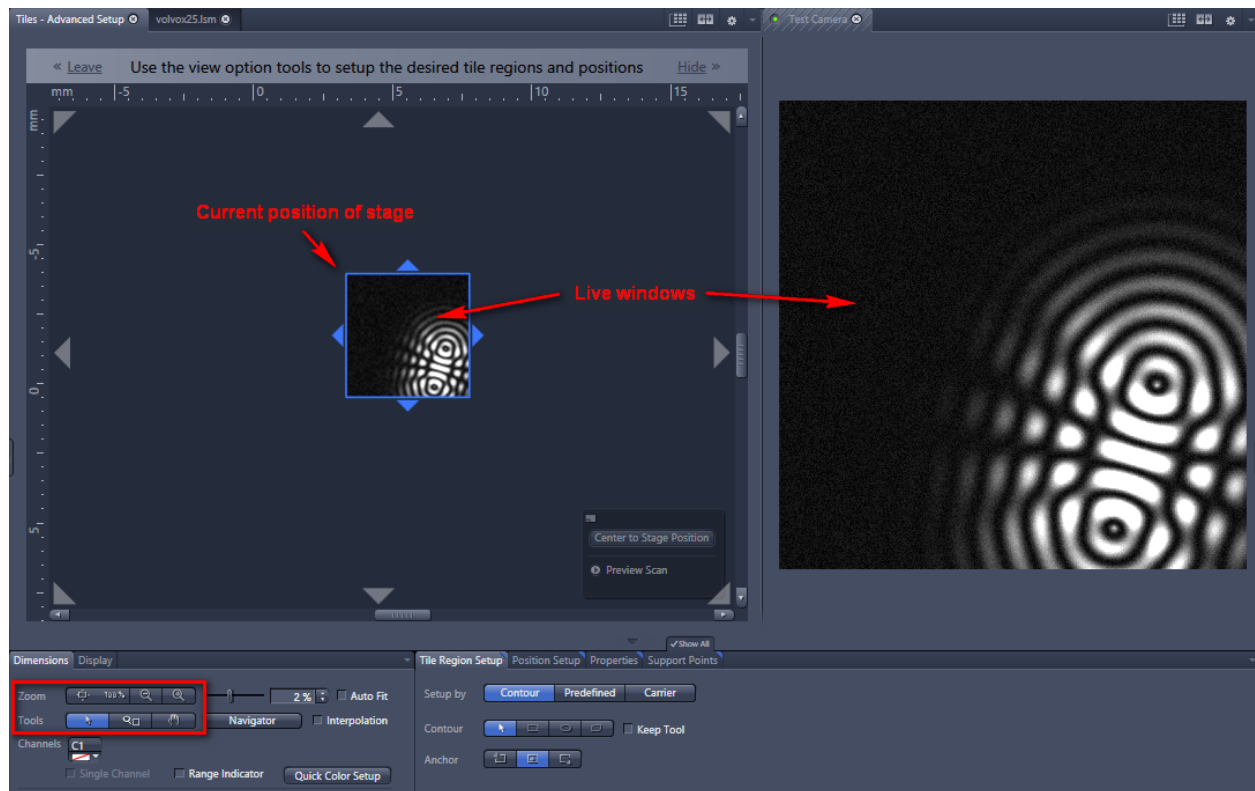
Expand the “Tiles” window that appears.

Expand the “Tile Regions” and “Positions” subsections and make sure that there are no existing tile regions or positions listed. If there are existing regions or positions, use the “gear” menu to “delete all” items in the list.

Activate the “Live in Separate Container” checkbox to ease locating and focusing regions in the following steps.

Click on the large “Advanced Setup” window.

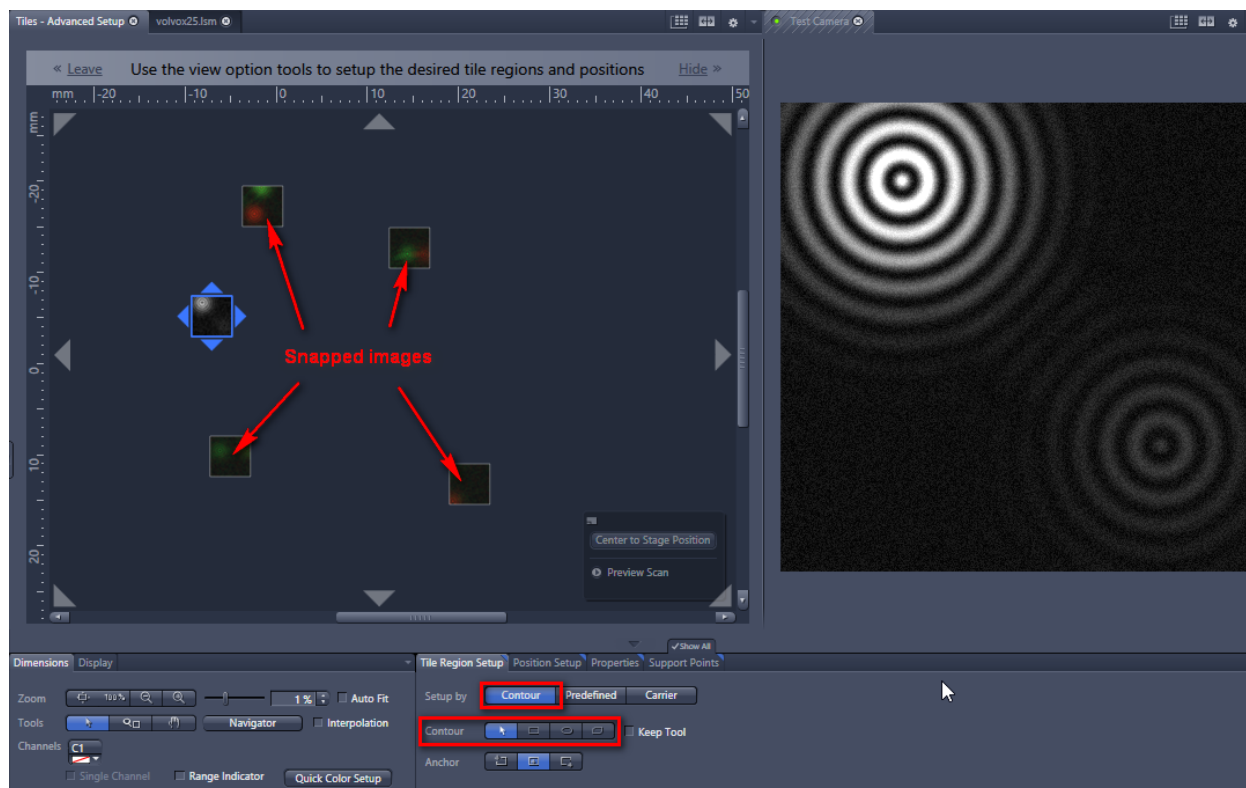




The “Advanced Setup” opens two containers. The left container represents the current stage position that the camera “sees”, outlined in blue and the rest of the sample field in the dark grey area. The right container displays a larger view of the live window from the currently selected channel.

Use the Zoom controls on the bottom left corner of the workspace to zoom in, zoom out and pan around the sample field. When the stage is moved with the joystick, the live view surrounded by the blue outline moves in relation to the sample field. You can also double-click anywhere within the sample field to move the stage to that location.

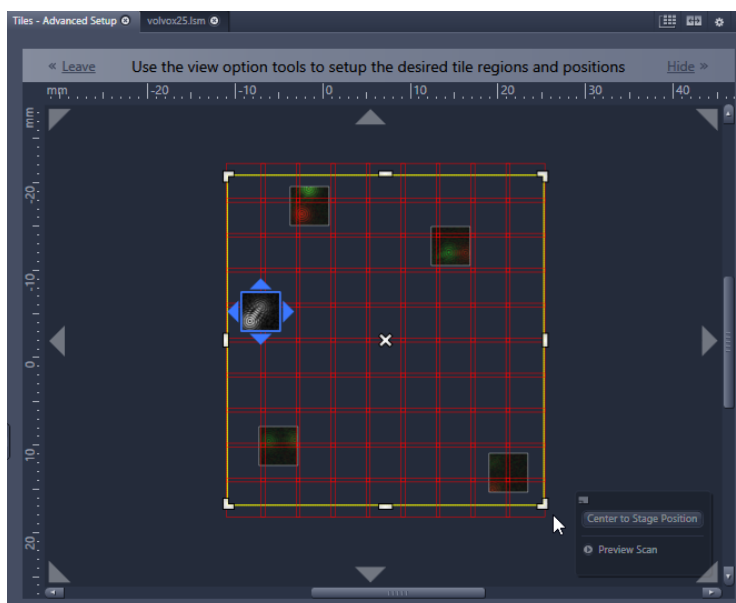
In addition to using the Zoom controls, you can spin the mouse wheel to zoom in and out. Click and hold down the mouse wheel (or middle button) to drag the sample field around (hand tool). If the live view surrounded by the blue outline gets lost, you can click the “Center to Stage Position” button in the translucent control window on bottom right corner of the sample field to relocate it.



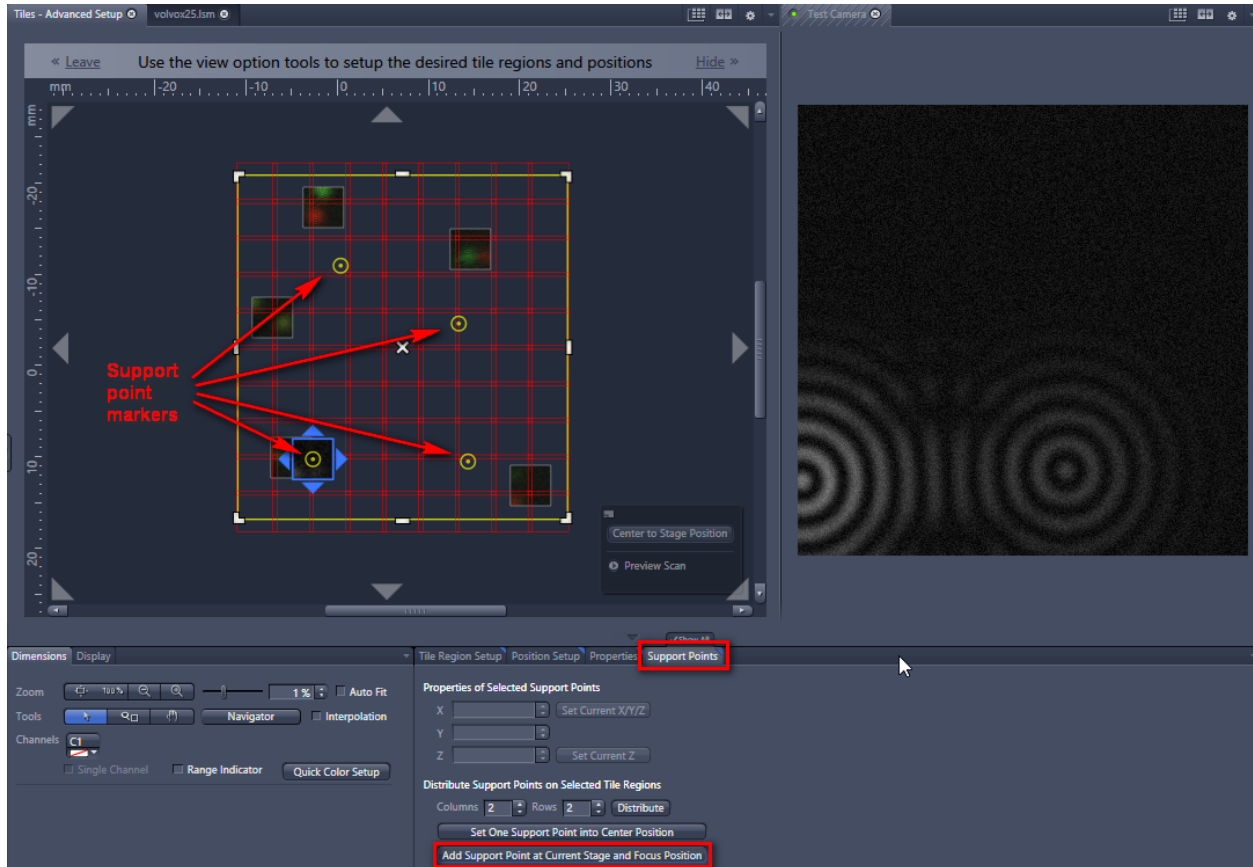
Move the joystick or double-click around the sample field to find the “edges” of the sample you wish to tile. At each “edge”, press the “Snap” button or hit “F2” to take an image at that position. These will serve as landmarks to help define the region you wish to tile.

When enough landmarks have been created to gain a general sense of the boundaries of the sample, create the tile region using the tools in the “Tile Region Setup” tab on the bottom of the screen. “Contour” will allow you to define the region by drawing an ROI using the tools provided.

After selecting an ROI tool, click and drag the tile region directly on the sample field making sure that all the landmarks have been included within the region. The red tiles indicate how many fields will be acquired using the current objective. The yellow border is the tile region you just drew. You can resize the tile region by grabbing and resizing the white handles. Changing objectives will automatically re-calculate the number of tiles necessary to acquire the tile region.



### 3. Adding support points to create a “Local Focus Surface”



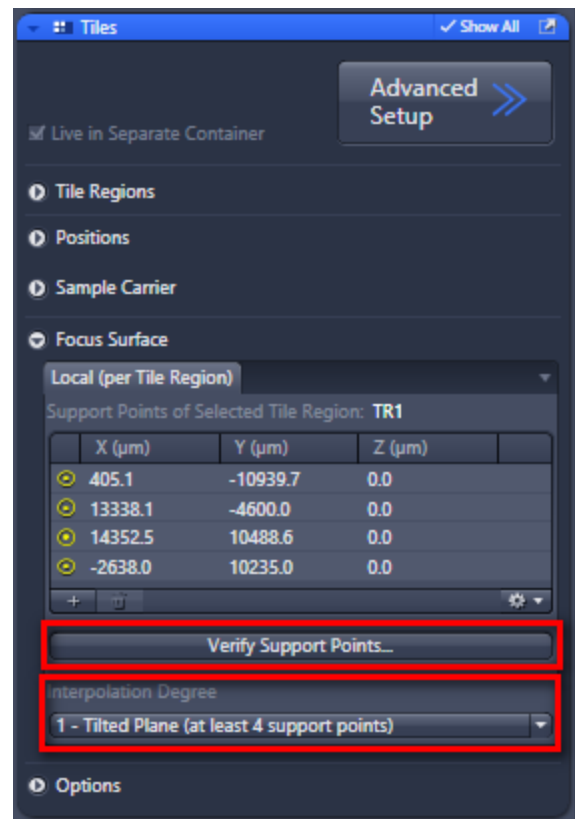
To create an interpolated focus surface (or map) to eliminate any focus variations in the sample, you need to create “Support Points”. These points will be used to calculate the focus surface. The more points you create, the more accurate the focus will be at any point within the tile region.

Start by double-clicking and moving the stage to various points within the tile region. At each position, focus critically using the live window in the right-hand container and press the “Add Support Point at Current Stage and Focus Position” button in the “Support Points” tab at the bottom of the screen.

Back in the “Tiles” window, expand the “Focus Surface” subsection to view the support points you created. The support points of the currently selected tile region are displayed.

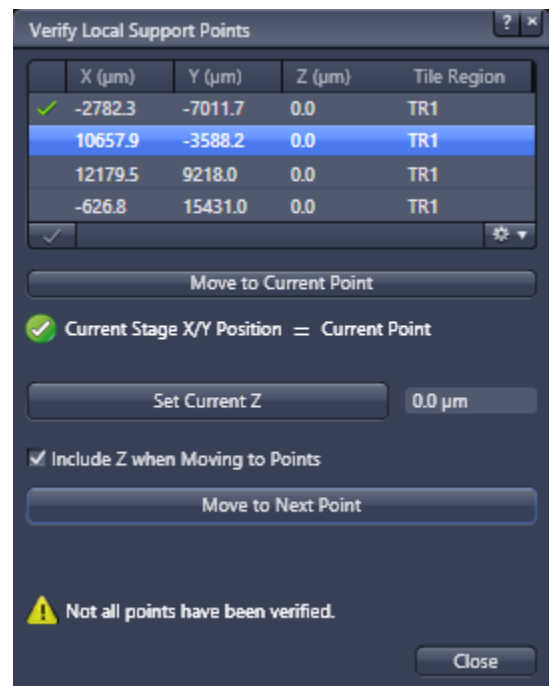
Select “1 – Tilted Plane” under the “Interpolation Degree” section to define a basic, interpolated focus surface.

If you would like to review and adjust the focal positions of the support points, press the “Verify Support Points...” button.



In the “Verify Local Support Points” window, highlight the first support point in the list and press “Move to Current Point”. Using the live window, focus critically at the support point’s position. Press the “Set Current Z” button to update the support point. Press the “Move to Next Point” button and repeat, focusing all remaining support points.

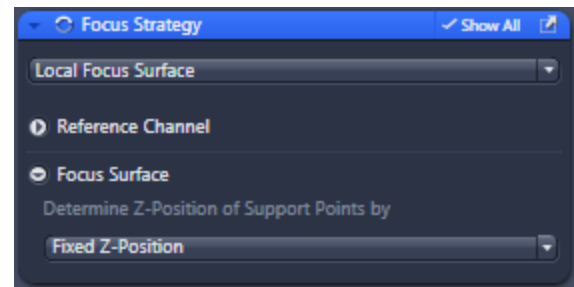
When you have verified all the focus support points, press the “Close” button.





#### 4. Activating the Focus Strategy and acquiring the tiled image

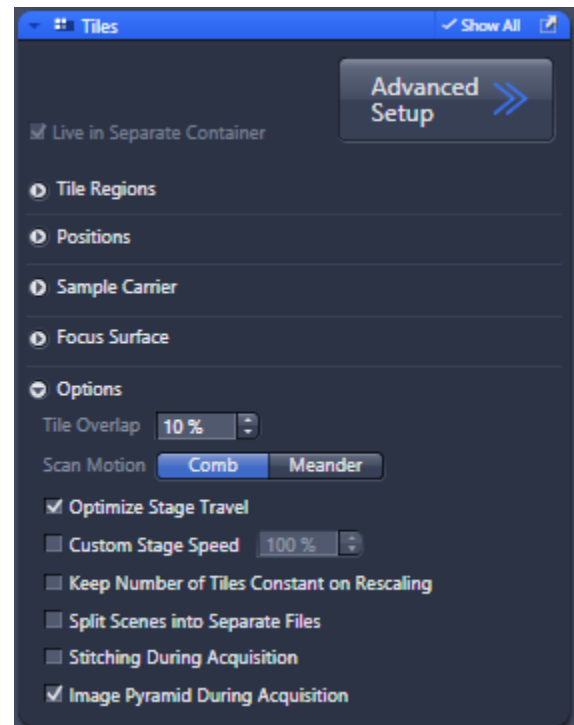
In the “Focus Strategy” window, select “Local Focus Surface” from the drop-down menu to activate and use the focus surface created in step 3.



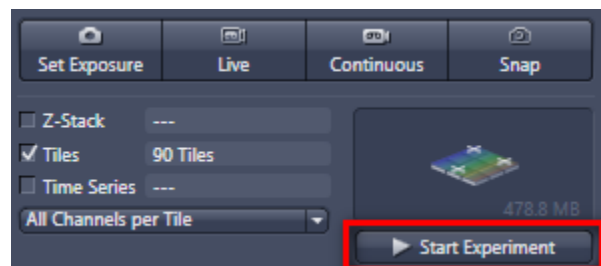
In the “Tiles” window, expand the “Options” subsection.

Make sure that a minimum “Tile Overlap” of 10% is defined. This will provide some imaging overlap between the edges of each tile so that the stitching algorithms will have enough correlation data to determine their correct positions in the final output.

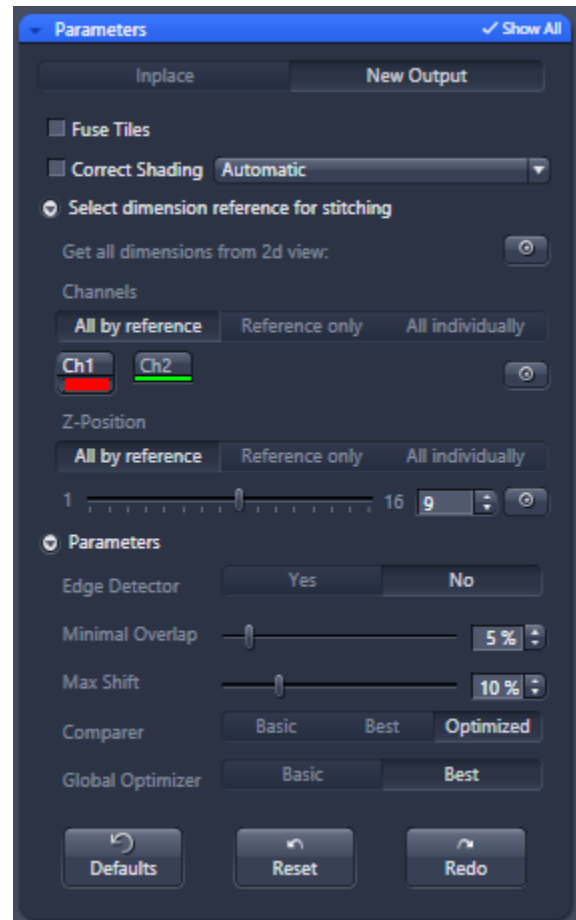
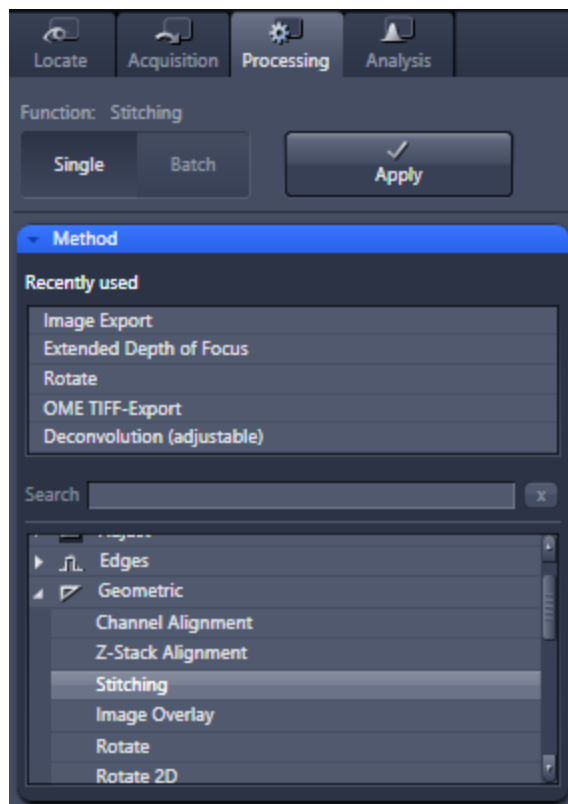
Check “Image Pyramid During Acquisition” to create the data necessary to open large tile images without consuming large amounts of system RAM.



Press the “Start Experiment” button to acquire the tiled image.



## 5. Stitching the tiled image



After the tile region has been acquired, navigate to the “Processing” tab and select “Stitching” under the “Geometric” subsection. The current image selected in the image container will be used for stitching.

Under the “Parameters” window, select “New Output”. Leave the “Fuse Tiles” and “Correct Shading” options unchecked. These options manipulate the image data and may cause certain artifacts to appear in the final image.

If you have collected multiple channels, it is important to just use one reference channel for the stitching. The tiles in the other channels will shift according to the reference channel. Select “All by reference” and choose the channel to perform the stitching algorithm on. It is best to choose a channel that has homogeneous signal throughout the tiled region.

If you have collected a Tile + Z-stack dataset, the same referencing image applies. Choose the z-plane that you would like to perform the stitching on.

Keep all other parameters in their default state. You may want to turn on the “Edge Detector” if the stitching is not accurate without it.

Press the large “Apply” button at the top of the screen to perform the stitching. A new window with the stitching results will appear after it has been processed.

